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Regular Article

Clinicopathological Significance and Prognostic Role of High Mobility Group Box 1 (HMGB1), Toll-Like Receptor (TLR) 2 and TLR4 in Breast Cancer

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High-mobility group box 1 (HMGB1) functions as damage-associated molecular pattern (DAMPs), released into extracellular space during cellular stress. Extracellular HMGB1 act as signal molecules through Toll-like receptor (TLR) 2 or TLR4, exerting diverse functions in both normal cells and malignant cells including breast cancer. However, their comprehensive examination in breast cancer tissues is lacking. Thus, we immunolocalized them in 112 breast cancer tissues, correlating their immunoreactivity with clinicopathological parameters and clinical outcomes to clarify their significance in breast cancer. We demonstrated that nuclear HMGB1 immunoreactivity was correlated with tumor progression and longer disease-free survival. In contrast, TLR2 immunoreactivity was correlated with increased cell proliferation and shorter disease-free survival, dependent on cytoplasmic HMGB1 immunoreactivity. Additionally, TLR4 immunoreactivity correlated with chemoresistance, regardless of cytoplasmic HMGB1 immunoreactivity. It was therefore considered that TLR2 collaboratively contributed to breast cancer progression with HMGB1-DAMPs to become a worse prognostic factor. Meanwhile, TLR4 served as a worse prognostic factor associated with chemoresistance, irrespective of HMGB1.

Key words: breast cancer, HMGB1, Toll-like receptor, immunohistochemistry, prognostic factor

I. Introduction

Breast cancer is one of the most commonly diagnosed cancers in females, representing 25% of new cases. Although recent studies have revealed the biological characteristics of breast cancer, leading to improvements in treatment such as endocrine therapy or chemotherapy, locoregional or distant metastases are still frequently observed. Therefore, exploring recurrence mechanisms and identifying novel biomarkers as potential therapeutic targets for breast cancer is crucial.

High-mobility group box 1 (HMGB1) is a prevalent damage-associated molecular pattern (DAMP), typically residing inside cells but released into the extracellular space during cellular stress [21]. HMGB1, which is initially localized in the nucleus, translocates to cytoplasm and then released into extracellular space. Once HMGB1 is released, it can bind to several surface receptors on immune cells to activate downstream intracellular signaling, triggering inflammatory response such as activation of innate immune

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cells and production of proinflammatory cytokines [12, 34]. Accumulating studies have indicated the importance of dysregulated HMGB1 signaling in tumorigenesis and HMGB1 is closely associated with cancer hallmarks [7], promoting proliferation, angiogenesis, altered energy metabolism while inhibiting anti-cancer immune system [12]. Recently, HMGB1 has been reported to be predominantly expressed in breast cancer and cytoplasmic HMGB1 is particularly considered to reflect the activation of HMGB1 as DAMPs [11, 16]. Examining the role of HMGB1 in breast cancer recurrence is important, as increased proliferation, invasion or angiogenesis is closely linked to the efficacy of endocrine therapy and chemotherapy of breast cancer.

Signal transduction of HMGB1 is mediated by membrane receptor such as Toll-like receptor (TLR) 2 and TLR4 [39]. Although TLR2 and TLR4 are predominantly expressed in antigen-presenting cells such as macrophages, recent studies indicate their expression in carcinoma cells and normal epithelium. TLR2 and TLR4 are considered to have pivotal roles in the progress of cancers either by inducing epithelial-mesenchymal transition (EMT) and invasion or inhibiting apoptosis, *via* crosstalk with other growth factor signaling [13, 23]. Aberrant expression of TLR2 or TLR4 has been reported in human malignancies. For example, TLR4 expression is associated with gastric cancer progression [40]. On the other hand, TLR2 and TLR4 are overexpressed in colorectal cancer, acting as adverse prognostic factors [2]. The clinical significance of TLR2 and TLR4 has also been suggested in breast cancer [22, 36].

Although previous findings suggest the involvement of HMGB1, TLR2, and TLR4 in breast cancer progression, they have not been comprehensively examined in breast cancer tissues. Therefore, we immunolocalized HMGB1, TLR2 and TLR4 in breast cancer tissues and corrected their immunoreactivities with clinicopathological parameters and clinical outcomes to elucidate their significance.

II. Materials and Methods

Patients and tissues

112 specimens of invasive breast cancer were obtained from the patients who had received surgical resection at Tohoku University Hospital from 2007 to 2008. All samples had been fixed by 10% formalin neutral buffer solution and embedded in paraffin. 90 patients had received adjuvant endocrine therapy, and 57 patients had received either neoadjuvant, adjuvant chemotherapy, or both. Clinical outcomes were evaluated by disease-free survival (from surgery to locoregional or distant metastasis) and median follow-up period was 59 months (3–84 months). This study was approved by the Ethics Committee at the Tohoku University School of Medicine.

Immunohistochemistry

Antibodies against HMGB1, TLR2 and TLR4 were purchased from Abcam (EPR35.7; Abcam, Cambridge, UK), NSJ Bioreagents (SanDiego, CA, USA) and Abnova (clone 1H7; Taipei, Taiwan), respectively. Antigen retrieval for HMGB1 and TLR4 were conducted by autoclave (121°C, 10 min, citrate buffer (pH 6) for HMGB1 and Tris/EDTA (pH 9) for TLR2, respectively). The antigen-antibody complex was visualized using Histofine Kit (Nichirei) and 3,3'-diaminobenzidine (DAB) [14], followed by counterstaining with hematoxylin. Human kidney, spleen and placenta were employed as positive controls for HMGB1, TLR2 and TLR4, respectively. For negative control, PBS was applied instead of primary antibody, confirming no significant staining on the slides. The immunohistochemical statuses of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 labeling index (LI) were referred to the previous reports [26, 38].

Scoring of immunoreactivities

The cases with immunoreactivity in more than 10% of carcinoma cells were considered positive for HMGB1, TLR2 and TLR4 [26, 29, 30, 38].

Statistical analysis

Statistical analysis was performed using JMP pro 17.0.0 software (SAS Institute, Cary, NC, USA). The correlation between clinicopathological parameters and the immunoreactivity of HMGB1, TLR2 and TLR4 was examined through χ^2 test or Mann–Whitney U test. Survival curve was generated by Kaplan-Meier method and statistically assessed with log lank test. Uni- and multivariate analyses were conducted using proportional hazard model (COX). $P < 0.05$ was considered significant.

III. Results

Immunolocalization of HMGB1, TLR2 and TLR4 in human breast carcinoma tissues

HMGB1 immunoreactivity was observed in both the nucleus (HMGB1-N) and cytoplasm (HMGB1-C) of breast carcinoma cells (Fig. 1A–D), with predominant detection in the nucleus of normal breast epithelium (Fig. 1E). Interestingly, the breast carcinoma cells adjacent to necrotic foci were frequently positive for HMGB1-C (Fig. 1C). 68 patients (61%) and 49 patients (44%) were classified as positive for HMGB1-N and HMGB1-C, respectively, and 22 patients (20%) and 27 patients (24%) were classified as double negative and double positive for HMGB1-N and HMGB1-C, respectively. TLR2 (Fig. 1F) and TLR4 (Fig. 1H) immunoreactivity was observed in the cytoplasm of breast carcinoma cells, whereas it was nearly negligible in normal breast epithelium (Fig. 1G, I). 47 patients (42%) and 41 patients (37%) were classified as positive for TLR2 and TLR4, respectively.

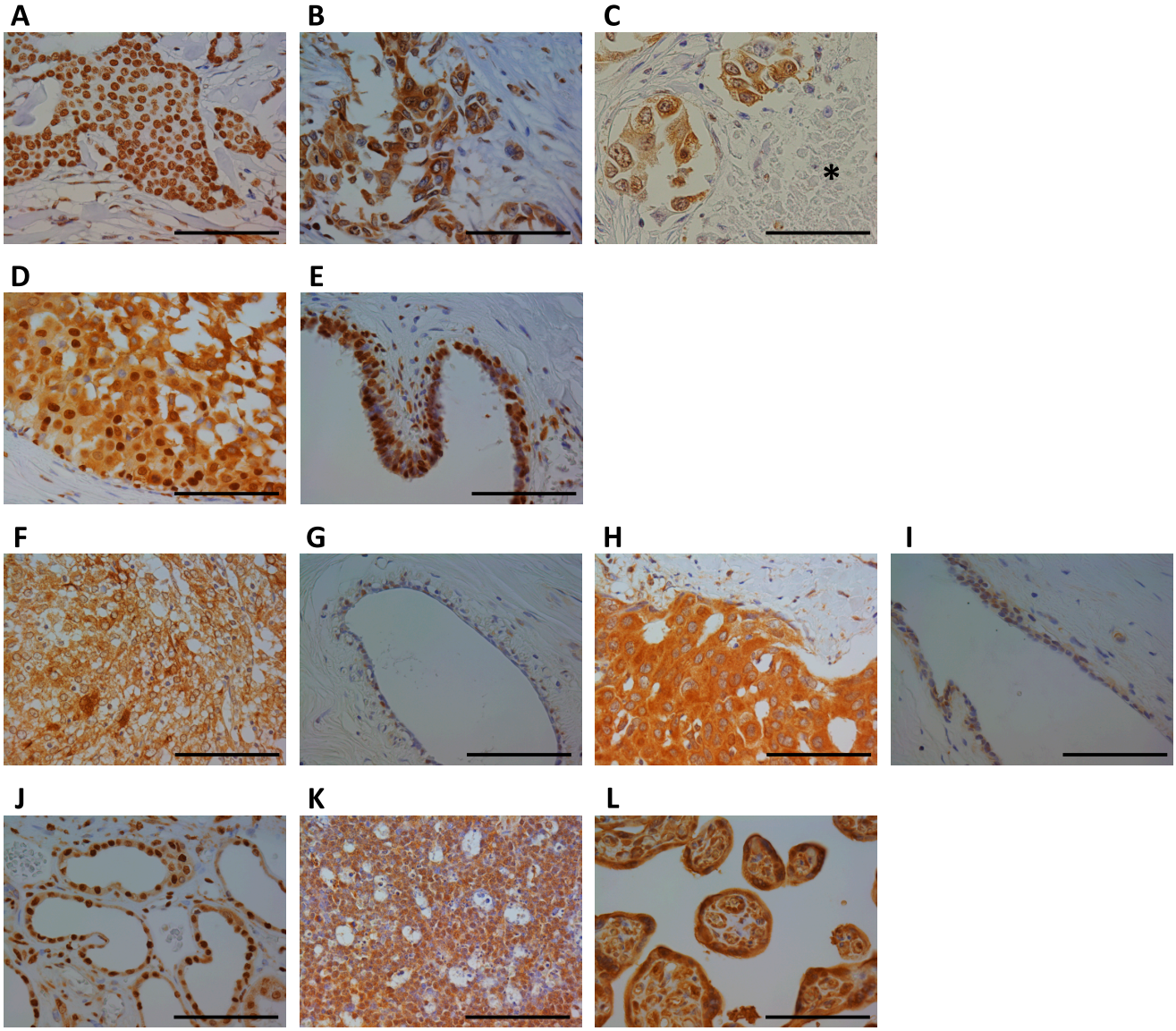

Fig. 1. Immunoreactivity of HMGB1, TLR2 and TLR4 in human breast carcinoma tissues. **A–E**; HMGB1 immunoreactivity was observed in the nucleus (**A**), cytoplasm (**B**, **C**) or both (**D**) of carcinoma cells, while it was predominantly detected in the nucleus of normal breast epithelium (**E**). *; necrotic focus. **F-I**; Immunoreactivity of TLR2 (**F**, **G**) and TLR4 (**H**, **I**) was observed in the cytoplasm of breast carcinoma cells (**F**, **H**), while negligible in normal breast epithelium (**G**, **I**). **J–L**; Positive control for HMGB1 (kidney; **J**), TLR2 (spleen; **K**) and TLR4 (placenta; **L**) immunostaining. Bar = 100 μm, respectively.

Association between clinicopathological parameters and immunoreactivity of HMGB1, TLR2 and TLR4 was summarized in Table 1. HMGB1-N immunoreactivity was inversely correlated with pathological T factor (pT, $P < 0.0001$), lymph node metastasis ($P < 0.0001$), stage ($P < 0.0001$), histological grade ($P = 0.0066$) and HER2 ($P = 0.020$) and the presence of necrotic foci ($P = 0.0004$), while it was positively correlated with PR ($P = 0.028$). In contrast, HMGB1-C immunoreactivity was inversely correlated with ER ($P = 0.035$), whereas positively correlated with necrotic foci ($P = 0.0002$).

TLR2 immunoreactivity was positively correlated with Ki67 LI ($P = 0.023$). Although P value did not reach to significant level, TLR4 immunoreactivity was positively correlated with pT ($P = 0.090$) and Ki67 LI ($P = 0.056$) and necrotic foci ($P = 0.0094$), whereas inversely correlated with PR ($P = 0.076$). Interestingly, HMGB1-C immunoreactivity tended to be correlated with TLR2 immunoreactivity ($P = 0.087$) and significantly correlated with TLR4 ($P = 0.017$). Additionally, significant positive correlation was detected between TLR2 and TLR4 immunoreactivity ($P < 0.0001$).

As HMGB1 is released from nucleus to extracellular space in response to stress, HMGB1-C might reflect the process of the activation of HMGB1 as a DAMP molecule. We further analyzed clinicopathological characteristics of

Table 1. *Clinicopathological characteristics of HMGB1, TLR2 and TLR4 in breast carcinoma tissues (n = 112)*

	HMGB1-N		P	HMGB1-C		P	TLR2		P	TLR4		P
	Negative (n = 44)	Positive (n = 68)		Negative (n = 63)	Positive (n = 49)		Negative (n = 65)	Positive (n = 47)		Negative (n = 71)	Positive (n = 41)	
Age*	57 (27–87)	55.5 (29–82)	0.39	57 (29–82)	56 (27–87)	0.92	58 (27–87)	56 (29–82)	0.37	56 (27–87)	56 (40–76)	0.75
Menopausal status												
Pre-	16	25	0.97	26	15	0.25	24	17	0.93	28	13	0.41
Post-	28	43		37	34		41	30		43	28	
pT												
pT1	17	57	**< 0.0001**	45	29	0.17	43	31	0.98	51	23	0.090
pT2-4	27	11		18	20		22	16		20	18	
Lymph node metastasis												
Negative	20	55	**< 0.0001**	44	31	0.46	43	32	0.83	51	24	0.15
Positive	24	13		19	18		22	15		20	17	
Stage												
1	13	50	**< 0.0001**	39	24	0.24	37	26	0.87	44	19	0.18
2	18	12		13	17		18	12		18	12	
3	13	6		11	8		10	9		9	10	
Histological grade												
1	10	34	**0.0066**	28	16	0.063	28	16	0.61	30	14	0.22
2	20	25		27	18		25	20		30	15	
3	14	9		8	15		12	11		11	12	
ER												
Negative	9	11	0.56	7	13	**0.035**	12	8	0.84	10	10	0.17
Positive	35	57		56	36		53	39		61	31	
PR												
Negative	19	16	**0.028**	18	17	0.49	22	13	0.49	18	17	0.076
Positive	25	52		45	32		43	34		53	24	
HER2												
Negative	33	62	**0.020**	53	42	0.82	54	41	0.55	62	33	0.33
Positive	11	6		10	7		11	6		9	8	
Ki67 LI*	13.5 (1–60)	11 (1–49)	0.65	9 (1–49)	14 (1–60)	0.11	8 (1–53)	14 (1–60)	**0.023**	9 (1–49)	16 (1–60)	0.056
Necrotic foci												
Negative	33	66	**0.0004**	62	37	**0.0002**	60	39	0.13	67	32	**0.0094**
Positive	11	2		1	12		5	8		4	9	
HMGB1-C												
Negative	22	41	0.28									
Positive	22	27										
TLR2												
Negative	24	41	0.55	41	24	0.087						
Positive	20	27		22	25							
TLR4												
Negative	24	47	0.12	46	25	**0.017**	55	16	**< 0.0001**			
Positive	20	21		17	24		10	31				

HMGB1-N; nuclear HMGB1, HMGB1-C; cytoplasmic HMGB1, LI; labeling index

*; data was presented as median (minimum-max).

All other values represent the number of cases. P < 0.05 was considered significant and described as bold.

TLR2 and TLR4 based on HMGB1-C status (Table 2) and found a significant correlation between Ki67 LI and TLR2 immunoreactivity in the HMGB1-C positive group (P = 0.039) but not in the HMGB1-C negative group (P = 0.38). Similarly, a significant negative correlation between PR and TLR4 was observed in the HMGB1-C positive group (P = 0.027) but not in the HMGB1-C negative group (P = 0.93). Furthermore, when the patients were categorized into two groups (i.e., HMGB1-C/TLR double positive group and others, Supplementary Table S1), HMGB1-C/TLR2

Table 2. *Clinicopathological characteristics of TLR2 and TLR4 according to cytoplasmic HMGB1 status*

	HMGB1-C negative group			HMGB1-C positive group			HMGB1-C negative group			HMGB1-C positive group		
	TLR2		P	TLR2		P	TLR4		P	TLR4		P
	Negative (n = 41)	Positive (n = 22)		Negative (n = 24)	Positive (n = 25)		Negative (n = 46)	Positive (n = 27)		Negative (n = 25)	Positive (n = 24)	
Age*	58 (33–82)	54.5 (29–82)	0.45	55.5 (27–87)	56 (40–76)	0.63	59 (29–82)	54 (42–76)	0.62	55 (27–87)	57.5 (40–75)	0.70
Menopausal status												
Pre-	16	10	0.62	8	7	0.69	19	7	0.99	9	6	0.40
Post-	25	12		16	18		27	10		16	18	
pT												
pT1	29	16	0.87	14	15	0.91	34	11	0.47	17	12	0.20
pT2-4	12	6		10	10		12	6		8	12	
Lymph node metastasis												
Negative	28	16	0.71	15	16	0.91	33	11	0.59	18	13	0.20
Positive	13	6		9	9		13	6		7	11	
Stage												
1	24	15	0.60	13	11	0.70	30	9	0.64	14	10	0.26
2	10	3		8	9		9	4		9	8	
3	7	4		3	5		7	4		2	6	
Histological grade												
1	19	9	0.23	9	7	0.35	20	8	0.67	10	6	0.45
2	15	12		10	8		21	6		9	9	
3	7	1		5	10		5	3		6	9	
ER												
Negative	6	1	0.22	6	7	0.81	6	1	0.42	4	9	0.088
Positive	35	21		18	18		40	16		21	15	
PR												
Negative	15	3	0.055	7	10	0.43	13	5	0.93	5	12	**0.027**
Positive	26	19		17	15		33	12		20	12	
HER2												
Negative	34	19	0.72	20	22	0.64	39	14	0.81	23	19	0.20
Positive	7	3		4	3		7	3		2	5	
Ki67 LI*	8 (1–49)	12.5 (1–44)	0.38	8 (1–53)	19 (1–60)	**0.039**	9 (1–49)	12 (1–44)	0.38	10 (1–49)	18.5 (1–60)	0.18
Necrotic fci												
Negative	41	21	0.17	19	18	0.56	46	16	0.097	21	16	0.16
Positive	0	1		5	7		0	1		4	8	

HMGB1-C; cytoplasmic HMGB1, LI; labeling index

*; data was presentesd as median (minimum-max). All other values represents the number of cases.

P < 0.05 was considered significant and described as bold.

double positive group were characterized by higher histological grade (P = 0.023) and higher Ki67 LI (P = 0.0065) and necrotic foci (P = 0.0037), while HMGB1-C/TLR4 group was characterized by ER/PR negativity (ER; P = 0.0046 and PR; P = 0.025) and higher Ki67 LI (P = 0.042) and necrotic foci (P = 0.0002).

Association between clinical outcome and immunoreactivity of HMGB1, TLR2 and TLR4

As shown in Fig. 2A, HMGB1-N immunoreactivity was significantly correlated with longer disease-free survival (P = 0.0001), while we did not detect a significant correlation between HMGB1-C immunoreactivity and disease-free survival (P = 0.41, Fig. 2B). On the other hand, both TLR2 (Fig. 2C) and TLR4 (Fig. 2D) immunoreactivity were correlated with shorter disease-free survival (TLR2; P = 0.0010 and TLR4; P < 0.0001). Moreover, TLR4 immunoreactivity seemed to be more strongly correlated with shorter disease-free survival in the patients who underwent chemotherapy (P = 0.0006) than those without chemotherapy (P = 0.049) (Supplementary Fig. S1).

We subsequently divided the patients according to HMGB1-C and TLRs. As shown in Fig. 2E, TLR2 immunoreactivity was significantly correlated with shorter disease-free survival in HMGB1-C positive group (P = 0.023), while no significant correlation was detected

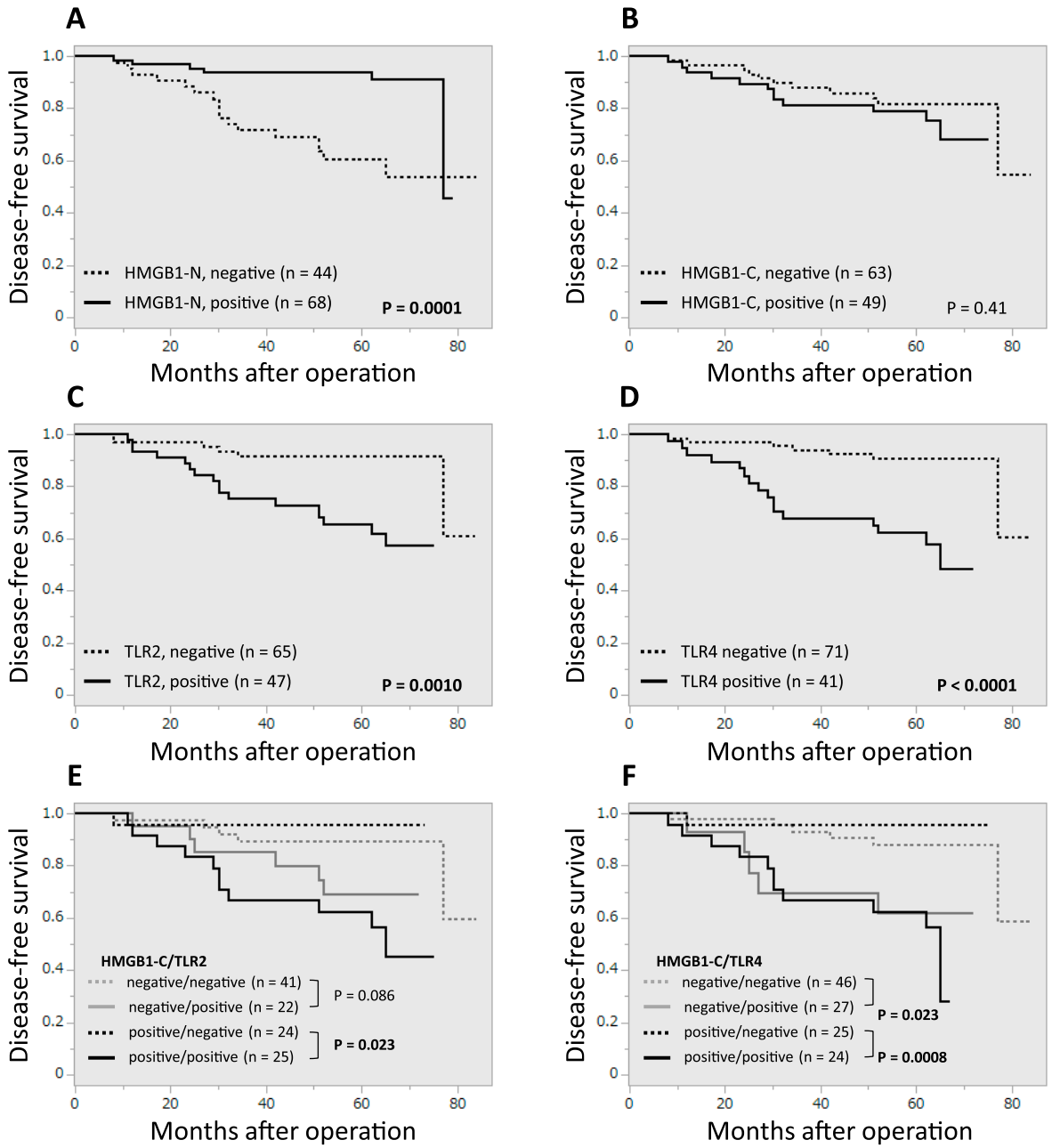

Fig. 2. Prognostic analysis according to HMGB1, TLR2 and TLR4. Kaplan-Meier curves according to nuclear HMGB1 (HMGB1-N; **A**), cytoplasmic HMGB1 (HMGB1-C; **B**), TLR2 (**C**) and TLR4 (**D**). The patients were divided into four groups according to the immunoreactivity of HMGB1-C as well as TLR2 (**E**) or TLR4 (**F**).

between them in HMGB1-C negative group (P = 0.086). In contrast, TLR4 immunoreactivity was significantly correlated with shorter disease-free survival regardless of HMGB1-C immunoreactivity (P = 0.023 in HMGB1-C negative group and P = 0.0008 in HMGB1-C positive group, Fig. 2F).

The results of univariate and multivariate analyses were summarized in Table 3. Univariate analysis revealed that pT, lymph node metastasis, histological grade, ER, PR, Ki67, necrotic foci, HMGB1-N, TLR2 and TLR4 were significant prognostic factors. Subsequent multivariate analysis revealed that only TLR2 (P = 0.010) was an independent prognostic factor for breast cancer-specific survival. Similarly, when HMGB1-C/TLR2 double positivity and HMGB1-C/TLR4 double positivity were considered instead of TLR2 and TLR4, respectively, only HMGB1-C/TLR2 double positivity (P = 0.034) was an independent prognostic factor (Supplementary Table S2).

Table 3. *Uni- and multivariate analysis of breast cancer-specific survival*

	Univariate		Multivariate	
	P	relative risk (95% CI)	P	relative risk (95% CI)
pT (pT2-4/pT1)	**0.0002**†	5.6 (2.3–14)	0.16	2.5 (0.70–8.9)
Lymph node metastasis (Positive/Negative)	**0.0027**†	3.6 (1.6–8.4)	0.42	1.6 (0.50–5.1)
Histological grade (3/1 + 2)	**0.0089**†	3.1 (1.3–7.1)	0.23	0.39 (0.0.082–1.8)
ER (Positive/Negative)	**0.0087**†	0.32 (0.13–0.75)	0.75	1.3 (0.0.31–5.2)
PR (Positive/Negative)	**0.0009**†	0.23 (0.097–0.55)	0.11	0.37 (0.11–1.2)
HER2 (Positive/Negative)	0.24	0.42 (0.098–1.8)		
Ki67 LI (≥20%/<20%)	**0.0005**†	4.5 (1.9–10)	0.051	3.6 (1.0–13)
Necrotic foci (Positive/Negative)	**0.0002**†	6.7 (2.8–16)	0.48	1.7 (0.38–7.7)
HMGB1-N (Positive/Negative)	**0.0006**†	0.20 (0.077–0.50)	0.11	0.38 (0.12–1.2)
HMGB1-C (Positive/Negative)	0.33	1.5 (0.66–3.5)		
TLR2 (Positive/Negative)	**0.0012**†	5.2 (1.9–14)	**0.010**	5.4 (1.5–20)
TLR4 (Positive/Negative)	**0.0002**†	5.9 (2.3–15)	0.47	1.6 (0.45–5.5)

HMGB1-N; nuclear HMGB1, HMGB1-C; cytoplasmic HMGB1, LI; labeling index

†; $P < 0.05$ was considered significant and exemined in the multivariate analysis.

IV. Discussion

In the present study, HMGB1 immunoreactivity was observed in both the nucleus and cytoplasm of breast carcinoma cells. In addition, HMGB1-N negatively correlated with the presence of necrotic foci, while HMGB1-C was positively correlated. This observation might indicate that HMGB1 is released into extracellular space necrotic tissues, where the breast cancer cells are exposed to cellular stress such as hypoxia. Clinicopathological characteristics of HMGB1-N and HMGB1-C were, however, not always consistent with previous reports. We demonstrated that HMGB1-N was correlated with less aggressive phenotype of breast cancers, whereas it had not been correlated with any clinicopathological parameters in the previous study [16]. On the other hand, in the present study, HMGB1-C was negatively correlated ER positively correlated with histological grade (although not statistically significant), which was consistent with previous study [16]. However, it has been also reported that HMGB1-C was correlated with smaller tumor size, lower pT and hormone receptor positivity [1]. These discrepancies may stem from differences in antibodies or the evaluation method of immunoreactivity. Nevertheless, it is intriguing that HMGB1 may exert different effects based on its localization. Furthermore, HMGB1-C was detected in carcinoma cells but not in normal breast epithelium, suggesting the specific function of HMGB1 in the cytoplasm of breast carcinoma cells and its potential reflection of DAMPs activity.

To the best of my knowledge, this is a first study demonstrating the clinicopathological characteristics of TLR2 immunoreactivity. While clinicopathological characteristics of TLR4 have been previously examined by several groups [6, 18, 19, 22, 35], we have provided the first evidence of the correlation between TLR4 and Ki67, a widely used biomarker to evaluate the proliferative index of breast cancer cells [9]. In this study, TLR2 immunoreactivity was significantly correlated with higher Ki67, while TLR4 tended to be correlated with higher Ki67. Additionally, pro-proliferative function of TLR2 and TLR4 in human cancer has been previously investigated through *in vitro* and *in vivo* analysis. For instance, miR-143 downregulates TLR2 expression and suppresses the growth and invasion of hepatoma cells [17]. Moreover, the TLR2 inhibitor, robinin has been reported to suppress the growth of pancreatic cancer by regulating TLR2-PI3K-AKT pathway [41]. On the other hand, TLR4 has been reported to promote the growth of *TP53*-mutant breast cancer cells [8]. Taken together, TLR2 and TLR4 are considered to contribute the growth of breast cancer.

Although we did not detect significant correlation between HMGB1 itself and aggressiveness of breast cancer, HMGB1 as a DAMP molecule is considered to promote the proliferation of human malignant cells [43]. We therefore analyzed clinicopathological characteristics of TLR2 and TLR4 according to HMGB-C status and demonstrated that TLR2 was correlated with higher Ki67 only in HMGB1-C positive group, while TLR4 did not show a correlation with Ki67 when the patients were divided according to HMGB1-C status. This finding might suggest that TLR2 serves as a receptor of HMGB1, and pro-proliferative role of TLR2 is dependent of HMGB1, while Other DAMPs than HMGB1 such as heat shock protein (HSP) 60, HSP70 and hyaluronan, may be associated with TLR4 activation [4].

In the present study, we for the first time demonstrated that TLR2 immunoreactivity was significantly correlated with shorter disease-free survival in patients, which aligns with a previous report utilizing quantitative PCR data [36] and microarray database [5]. This observation might be partly explained by the expression of TLR2 in cancer stem cells [3], and increased stemness has been associated with therapeutic resistance and worse clinical outcomes in breast cancer [20]. Notably, TLR2 immunoreactivity was correlated with shorter disease-free survival only in the HMGB1-C positive group. Therefore, it is considered that TLR2 contributed to breast cancer progression in concert with HMGB1 as DAMPs.

We also detected significant relationship between TLR4 immunoreactivity and worse clinical outcome.

Although a previous study has failed to detect the correlation between TLR4 immunoreactivity and prognosis of breast cancer patients [22], this discrepancy may stem from differences in positive rates due to varying methods of TLR4 immunostaining (37% in the precent study, while 90% in the previous study). It is noteworthy that therapeutic resistance plays a significant role in the clinical outcome of breast cancer. While endocrine therapy is utilized in adjuvant therapy for less aggressive breast cancer, cytotoxic chemotherapy is often used either as a standalone therapy or in combination with endocrine therapy for highly aggressive breast cancer, including triple-negative breast cancer. In this study, TLR4 immunoreactivity was correlated with the overall outcome regardless of HMGB1; however, TLR4 exhibited a stronger correlation with a worse prognosis in patients who had received chemotherapy compared to those without chemotherapy. Moreover, previous *in vitro* studies have indicated the contribution of TLR4 to chemoresistance in human cancers, including breast cancer, prostate cancer, ovarian cancer, and hepatocellular carcinoma [10, 28, 31, 33, 42]. As possible mechanisms of TLR4-mediated chemoresistance, TLR4 has been reported to be directly activated by paclitaxel and recruit endothelial progenitor cells, inducing lymphatic or hematogenous metastasis [24]. Moreover, activation of TLR4 causes induction of *ABCB1* (ATP-binding Cassette Sub-family B Member 1), an ATP-dependent drug efflux pump protein [27] in ovarian cancer. On the other hand, activation of TLR4 induces mesenchymal phenotype and stemness property of breast cancer cells [32], which is closely associated with chemoresistance [25]. Taken together, TLR4 is considered to contribute to chemoresistance independently of HMGB1 and serve as a worse prognostic factor in breast cancer.

Prognostic role of HMGB1 has been examined in various human malignancies, and HMGB1 is generally considered as a worse prognostic factor in human malignancies like gastric, colon, pancreatic and cervical cancer [37]. However, it remains controversial either nuclear or cytoplasmic HMGB1 serves as a prognostic factor in breast cancer, as opposite findings have been reported by several researchers [1, 15, 16]. Nevertheless, we observed a significant correlation between HMGB1-N and longer disease-free survival. These findings may reflect the complex biological functions of HMGB1 in human malignancies [12], and the role of HMGB1 may be influenced by other factors, such as TLRs.

V. Conflicts of Interest

The authors have no conflict of interest in this study.

VI. Acknowledgments

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